# REMARKS

## Status of the Claims

Claims 1-34, and 36-43 are pending and under consideration in this application, claim 35 having been cancelled without prejudice to it being prosecuted in a separate application. All the pending claims stand rejected.

## Sequence Listing

In response to the comments on page 2, lines 2-8, of the Office Action, and the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures (Notice to Comply") attached to Office Action, Applicants submit herewith, under 37. C.F.R. §§1.821-1.825, a Sequence Listing in hard copy and in computer-readable form and a Copy of the Notice to Comply.

## 35 U.S.C. §112, second paragraph, rejection

Claims 1-34, 36, and 37 stand rejected as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants respectfully traverse this rejection.

Applicants submit that the comments on page 2, lines 16-18, are moot in light of the definition (on page 2, lines 14-19, of the specification) of "significant binding affinity" as used in claim 1.

While Applicants do not agree that claim 23 is unclear as written, in order to expedite prosecution of the instant application, Applicants have amended the claim to further clarify it. For the sake of consistency, claim 39 has been similarly amended. The amendments to claims 23 and 39, which do not narrow the scope of the claims, is supported by the specification, e.g., at page 18, lines 10-11.

In regard to the comments on page 3, lines 5-7, of the Office Action, Applicants have corrected the dependency of claim 34 in order to provide the appropriate antecedent basis for the term "said cell population." The amendment, which does not narrow the scope of the claim, is supported by the specification, e.g., at page 5, lines 6-11.

In light of the above remarks and amendments, Applicants request that the rejection of the claims under 35 U.S.C. §112, second paragraph, be withdrawn.

35 U.S.C. §112 first paragraph, rejections

(a) Claims 1-43 stand rejected on the grounds that the specification allegedly does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. Applicants respectfully traverse this rejection.

Claim 35 is cancelled.

From the comments on page 4, line 15, to page 6, line 4, of the Office Action, Applicants understand the Examiner's position to be that the references cited in the text support the idea that vector-targeting is the major obstacle to the success of gene therapy. Applicants submit that while it may be true that, at least in some systems, vector-targeting remains a problem for *in vivo* methods or gene therapy, the references cited by the Examiner support the idea that this obstacle is either absent or substantially minimized in *ex vivo* methods of gene therapy.

Thus, for example, Miller et al. states:

> Of the gene therapy protocols that have so far entered clinical trials, targeting of the appropriate vectors has been achieved largely by indirect means. Thus, several such trials (for example, for treatment of ADA deficiency, HIV infection, or cancer) have used specific cell populations that have been removed from the patient and infected in vitro by nontarget amphotropic retroviruses before being returned in vivo. (page 197, column 2, paragraph 2)

Of particular relevance to the instant application, Miller et al. states in addition that:

> further levels of targeting have been achieved in some cases by careful choice of the patient's cells; for instance, ex vivo production of tumor infiltrating lymphocytes with potentially tumoricidal genes has been proposed as a means of delivering their products to tumor deposits at much higher concentrations than would otherwise be possible. (page 197, column 2, paragraph 2)

Miller et al. also points to an advantage of *ex vivo* over *in vivo* methodologies in that in "ex vivo manipulation of target cells . . . the vector requires very little, if any, intrinsic targeting capability." (page 197, column 2, paragraph 3)

Deonarain does not describe targeting cells of the type used in the instant invention whose functionality is based on their ability to bind, via interactions between receptors and ligands on the targeting and target pathogenic cells, to a pathogenic target cell. However, Deonarain provides an extensive description of experiments in which receptor-ligand interactions were successfully exploited for the purpose of targeting expression vectors of choice to desired target cells (e.g., Abstract and page 57, column 2, paragraph 5, to page 65, column 1, paragraph 5). The "Expert Opinion" at the end of the article is optimistic regarding the approach (pages 65-66). In so far as the targeting cell methodology of instant invention depends on similar receptor-ligand interactions to those employed in the methods described by Deonarain, similar reasons for optimism apply to it.

In Verma et al., only two gene therapy systems (one experimental on page 240, column 1, last paragraph, to column 3, first line, and the other clinical on page 242, column 1, paragraph 2) were described in some detail as having been successful. Both systems involved *ex vivo* methods. Significantly also with regard to *ex vivo* methods, Verma et al. states "[a] critical limitation of retroviral vectors is their inability to infect non-dividing cells[8], such as those that make up muscle, brain, lung, and liver tissue. So, when possible, the cells from target tissue are removed, grown *in vitro*, and infected with the recombinant retroviral vector." (page 241, paragraph spanning columns 1 and 2)

Crystal reviews a large number of studies on human gene transfer. The large majority of those in which successful gene transfer were obtained involved *ex vivo* methods (page 405, column 3, paragraph 2). Moreover, a greater proportion of the studies in which appropriate biologic responses were obtained employed *ex vivo* methods (page 407, column 1, paragraph 3, to page 408, column 2, last paragraph). Of particular relevance to the instant invention are the impressive *ex vivo* studies described in Crystal carried out using T cells, and in particular, cytotoxic T cells (CTL) for gene delivery (page 408, column 2, paragraphs 2 and 3).

Thus, while the cited references do point out problem areas in gene therapy, they are all ultimately highly optimistic regarding the efficacy of gene therapy, and in particular, *ex vivo* gene therapy.

A major advantage of *ex vivo* over *in vivo* gene therapy methods is the ability to select or enrich for successfully transfected or infected cells prior to administration. Moreover, cells

successfully transfected or infected can be further selected on the basis of their ability to produce high levels of a relevant recombinant protein. These attributes of *ex vivo* gene therapy avoid the challenge of obtaining sufficient numbers of cells producing sufficient amounts of a recombinant protein inherent in *in vivo* methods. Applicants point out that, in addition to these advantages, the *ex vivo* methodology of the instant invention employs two "layers" of targeting that particularly enhance the chances of its success in terms of cell targeting, the aspect of gene therapy held by the Examiner to be particularly problematic (page 4, lines 17-19, of the Office Action). First, the targeting cells serve to direct the gene of interest to organ or tissue of interest. Second, once the gene product is produced by the targeting cells within the organ or tissue of interest, it is targeted to target cells of interest via its targeting domain. The targeting cells "home" to the tissue (e.g., tumor tissue) of interest by virtue of either (a) their antigen-specificity mediated by cell surface antigen-specific receptors (on, for example, T or B lymphocytes), and/or (b) tissue-specificity mediated by tissue-specific interactions between receptors and ligands on the targeting cells and target cells in the tissue (or other cells in the vicinity of the target cells) (see, for example, page 15, line 28, to page 16, line 6, of the specification). The immunotoxic gene product secreted by targeting cell binds to a target cell by receptor-ligand-type interactions between the its targeting domain and a cell surface component on the target cell (e.g., page 19, line 6, to page 23, line 14, of the specification).

With respect to the remarks on page 8, lines 3-13, of the Office Action, Applicants submit that one of skill in the art would likely know what range of cell surface receptors, ligands, etc. are expressed on target cells and potential targeting cells of interest. Alternatively, if such an artisan was not in possession of this information, she would be aware of: (a) how to "search" the medical or scientific literature; or (b) the routine experimentation to perform in order to decide upon a suitable molecule for use as a targeting domain.

With regard to administration, Applicants point out that, as indicated on page 36, line 32, to page 37, line 1, of the instant specification, administration of the targeting cells of the invention can accomplished "either at the site of the disease or systemically." As pointed out above, an advantage of the instant invention is that the targeting cells have the property to home to and thereby to concentrate at the disease site, Thus, contrary to the assertion on page 9, lines 10-13, of the Office Action, the targeting cells can be administered systemically. Indeed, in the

experiment described in Example 6, inhibition of tumor growth in the flanks of mice was obtained by systemic (intravenous) injections of the relevant targeting cells.

In light of the above considerations, Applicants submit that one skilled in the art could make and use the invention without undue experimentation.

(b) Claims 1-43 stand rejected as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention. Applicants respectfully traverse the rejection.

As pointed out above, claim 35 has been cancelled.

From the comments on page 9, line 9, to page 11, line 15, of the Office Action, Applicants understand the Examiner's position to be that it in order to fulfill the written description requirement, it is necessary that Applicants provide the nucleic acid sequences of all the fusion-protein encoding constructs (or the amino acid sequences of such fusion proteins) used in the vectors, targeting cells, and methods that would be encompassed by the invention (see particularly page 9, lines 9-14, and page 1, lines 7-10, of the Office Action). Applicants respectfully submit that there is no absolute requirement that proteins, and in particular fusion proteins, and the nucleic acids encoding them be described in terms of sequence. Thus, for example, a recently issued U.S. Patent (No. 6,342,345 (the '345 patent), issued January 29, 2002) broadly claims (a) a reporter system component that in one embodiment is a fusion protein, and (b) a nucleic acid encoding such a fusion protein. No amino acid or nucleic sequences were provided for any of the fusion proteins covered by the claims. As in the instant application, the representative polypeptides listed as being useful components of fusion protein (for the purposes of the '345 patent) were known polypeptides. Applicants also draw the Examiner's attention to other examples of recently issued U.S. patents in which even novel polypeptides were claimed without reference to amino acid or nucleic acid sequences (e.g., U.S. Patent Nos. 5,883,227, issued March 16, 1999; 5990,283, issued November 23, 1999; and 6,140,467, issued October 31, 2000).

Applicants respectfully submit that the fusion proteins encoded by the vectors of the invention and secreted by the targeting cells of the invention are adequately described in the

instant specification. First, they are structurally defined in terms of the components required. With respect to the generic claims, multiple representative examples of previously known polypeptides useful as targeting domains and toxic domains are listed in the specification (see, for example, page 19, line 18 to page 23, line 14 and page 23, line 19, to page 24, line 21, respectively). In addition, even though not required (because all the listed representatives were known at the time of filing of the instant application), references containing nucleic sequences encoding many such polypeptides are provided by the specification (e.g., page 22, line 15, to page 23, line 5, and page 24, lines 10-21, of the specification).

As indicated on page 17, lines 28-30, of the specification, linker sequences are not required in the fusion proteins produced by the targeting cells and encoded by the vectors of the invention. Indeed, as indicated in the Chan et al. (1996) reference cited by the Examiner and referred to in the instant specification (e.g., page 39, line 20), an effective immunotoxin composed of an IL-3 targeting domain and a DT390 toxic domain was produced without a linker. In addition, even though the amino acid sequences of useful linker sequences are known in the art, in addition to the linker specifically referred to in the specification (SEQ ID NO:3), a description of the structural requirements for linkers is provided on pages 18, lines 3-7, of the specification. Moreover, the Chan et al. (1995) reference cited by the Examiner and also referred to in the instant specification (e.g., page 38, lines 28-29) provided the amino acid sequence of another useful linker sequence (page 2735, column 1, paragraph 4).

Finally, even though signal peptides are known in the art, the amino acid sequence of one is specifically provided (SEQ ID NO:1 on page 18, line 31, of the specification) and another is described in sufficient detail to allow one skilled in the art to easily obtain the sequence (page 38, lines 21-24, of the specification).

With respect to species claims, the sequences of nucleic acids encoding IL-4, DT and all the polypeptides listed in claims 5, 19, and 31 are known and references containing the sequences of some are provided on page 22, line 15, to page 23, line 5, and page 24, lines 10-21, respectively, of the specification.

In addition to containing the relevant structural information, the specification and claims contain substantial information on the functional properties of the elements of the fusion proteins encoded by the vectors and secreted by the targeting cells of the invention. Such information is

provided at multiple sites on, for example, page 6, line 5, to page 7, line 16, and page 19, line 5, to page 24, line 21 of the specification. Some of the functional properties described in the specification are also recited in the claims, e.g., independent claims 1, 36, and 38. Finally, Applicants submit that there is a direct correlation between the function of the two domains of the fusion proteins and their structures. Thus, the toxicity of toxic domains is a direct consequence of their known amino acid sequences and hence also of the known nucleotide sequences of nucleic acids encoding them. In addition, the ability of a targeting domains to bind to appropriate receptors/ligands on target cells is a direct consequence of their known amino acid sequences and hence of the known nucleotide sequences of nucleic acids encoding them.

In light of the above considerations, Applicants submit that the instant specification contains adequate written description of the fusion proteins encoded by the vectors and secreted by the targeting cells of the invention.

In view of the above arguments, Applicants request withdrawal of the rejections under 35 U.S.C.§112, first paragraph.

35 U.S.C. §102(b) rejection

Claims 38-41, and 43 stand rejected as allegedly being anticipated by Chan et al. (Blood 86:2732-2740, 1995).

Applicants understand the Examiner's position to be that Chan et al. discloses vectors encoding fusion proteins containing a truncated diphtheria toxin molecule (DT390) and either GM-CSF, IL-2, IL-4, IL-6, or G-CSF. Applicants agree that Chan et al. mentions fusion proteins containing IL-2, IL-4, IL-6, and G-CSF (page 2732, column 2, last complete paragraph) and refers specifically to fusion proteins containing DT390 and either IL-2 or IL-4 (e.g., at page 2734, column 2, second paragraph). However, the reference does not disclose or even suggest how these fusion proteins were made, let alone disclose vectors containing nucleic acid sequences encoding the fusion proteins. While the fusion proteins may have been made by recombinant methods using expression plasmids, they were not necessarily made in this way. They could have been constructed using, for example, chemical methods of fusing proteins. Despite these considerations, in order to expedite prosecution of the instant application, Applicants have amended claim 38 to specify that the vector claimed is a viral vector. In

addition, claim 43 has been amended by deletion of the term "a plasmid" from line 2. These amendments are supported by the specification, e.g., at page 32, lines 1-6.

In light of the above amendments and remarks, Applicants submit that the claims are not anticipated by the cited art and respectfully request that the rejection under 35 U.S.C. §102(b) be withdrawn.

35 U.S.C. §103(a) rejection

Claims 1-33 and 36-43 stand rejected as allegedly being unpatentable over Chan et al. (Blood 86:2732-2740, 1995 and Blood 88:1445-1456, 1996) in view of Yang et al. (Nature Biotechnology 15:46-51, 1997) and further in view of Chen et al. (Nature 385:78-80, 1997). Applicants respectfully traverse this rejection.

From the comments on page 13, line 1, to page 14, line 9, of the Office Action, Applicants understand the Examiner's position to be that disclosure of the two Chan et al. references combined with that of Yang et al. and Chen et al. renders the above listed claims obvious. Applicants disagree with this position. The two Chan et al. references describe experiments with fusion proteins containing DT390 fused to either GM-CSF or IL-3. No mention or suggestion of the desirability of using targeting cells of any sort, let alone CD8+ CTL, to direct expression of a gene encoding an immunotoxic protein to a tumor cell or infected cell is made by either Chan et al. reference. Thus neither reference contains the necessary motivation to combine its disclosure with that of Yang et al. and/or Chen et al. and thereby to render obvious the invention specified by the instant claims.

Yang et al. and Chen et al. describe experiments using CD8+ CTL to target expression of genes encoding immunotoxins using antibody fragments (Fab and single chain Fv, respectively) fused to toxic proteins to HIV- infected cells *in vitro* and tumor cells *in vitro* and *in vivo*, respectively. The two references focus exclusively on the use of antibodies or antibody fragments for use as targeting domains in immunotoxins. There is in neither reference any mention or the suggestion of the desirability of using targeting domains other than antibodies or antibody fragments. Thus, neither Yang et al. nor Chen et al. contains the necessary motivation to combine its disclosure with that of either or both of the Chan et al. references. and thereby to render obvious the invention specified by the instant claims.

In light of the above considerations, Applicants respectfully request that the rejection under 35 U.S.C. §103(a) be withdrawn.

Attached is a marked-up version of the changes being made by the current amendment.

## CONCLUSION

In summary, for the reasons set forth above, Applicants maintain that all of the pending claims patentably define the invention. Applicants request that the Examiner reconsider the rejections as set forth in the Office Action and permit the pending claims to pass to allowance.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicants' undersigned representative can be reached at the telephone number listed below.

Enclosed is a petition for an automatic extension of time and check in payment of the extension. Please apply any additional charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 2/14/02

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**Version with markings to show changes made**

In the claims:

Claim 35 has been cancelled.

Claims 23, 38, 39, and 43 have been amended as follows:

23. (Amended) The targeting cell of claim 1, wherein said vector further comprises [comprising, 5' of the 5' end of said encoding sequence,] a mammalian signal sequence, wherein said mammalian signal sequence is located 5' of the 5' end of said nucleic acid sequence encoding the fusion protein.

38. (Amended) A viral vector comprising a nucleic acid sequence encoding a fusion protein, said fusion protein comprising:

(a) a targeting domain comprising a first member of an affinity pair;

(b) a toxic domain comprising a toxic molecule; and

(c) transcriptional and translational regulatory sequences operably linked to said DNA sequence, said regulatory sequences allowing for expression of said fusion protein in a cell of a mammal,

wherein said first member binds to a second member of said affinity pair, said second member being expressed on a surface of a pathogenic cell.

39. (Amended) The vector of claim 38, further comprising[, 5' of the 5' end of said encoding sequence,] a signal sequence, wherein said signal sequence is located 5' of the 5' end of said nucleic acid sequence encoding the fusion protein.

43. (Amended) The vector of claim 38, wherein the vector is selected from the group consisting of [a plasmid,] an adenoviral vector, a adeno-associated viral vector, a vaccinia viral vector, a lentiviral vector, and a herpes viral vector.

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Attorney Docket No.: 11983-004001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Daniel A. Vallera et al.
Serial No. : 09/579,738
Filed : May 26, 2000
Title : CELL-MEDIATED TARGETING OF TOXINS TO PATHOGENIC CELLS

Art Unit : 1643
Examiner : Unknown

Commissioner for Patents
Washington, D.C. 20231

VERIFIED STATEMENT UNDER 37 CFR §1.821(f)

I, Katica Magovcevic, declare that I personally prepared the paper and the computer-readable copy of the Sequence Listing filed herewith for the above-identified application and that the content of both is the same.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of The United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 2-14-02

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| Notice to Comply | Application No. | Applicant(s) | |
|---|---|---|---|
| | Examiner | Art Unit | |

# NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Applicant must file the items indicated below within the time period set the Office action to which the Notice is attached to avoid abandonment under 35 U.S.C. § 133 (extensions of time may be obtained under the provisions of 37 CFR 1.136(a)).

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

☒ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998).

☒ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).

☒ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).

☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable from of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).

☐ 7. Other:

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☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".

☒ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.

☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

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